PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Peter R. REEVES et al Confirmation No. **6333**

Appln. No.: **09/423,093** Group Art Unit: **1655**

Filed: **November 1, 1999** Examiner: **Sisson, B.**

For: **NUCLEIC ACID MOLECULES SPECIFIC FOR BACTERIAL ANTIGENS AND USES THEREOF**

AMENDMENT AFTER FINAL

Assistant Commissioner
of Patents
Washington, D.C. 20231

Sir:

This Amendment After Final is in response to the Office Action dated July 23, 2002, in the above-identified application for which a Petition for a Two-Month Extension of Time, along with payment of the appropriate fee, is attached, making response due on or before December 23, 2002.

The Patent Office is authorized to charge any fees necessary for the continued pendency of the above-identified application to our Deposit Account No. 19-4880.

Accordingly, please amend the above-identified application as follows.

IN THE SPECIFICATION:

Please amend the specification as follows:

Page 26, line 15 to 36, delete in their entirety.

Page 27, lines 1-3, delete in their entirety, and insert therefor

-- Chromosomal DNA was prepared from bacteria grown overnight at 37°C in a volume of 30 mL of Luria broth. After harvesting by centrifugation, cells were washed and resuspended in 10 mL of 50 mM Tris-HCl pH 8.0. EDTA was added and the mixture incubated for 20min. Then lysozyme was added and incubation continued for a further 10min. Proteinase K, SDS, and ribonuclease were then added and the mixture incubated for up to 2hr for lysis to occur. All incubations were at 37°C. The mixture was then heated to 65°C and extracted once with 8mL of phenol at the same temperature. The mixture was extracted once with 50mL of phenol/chloroform/iso-amyl alcohol at 4°C. Residual phenol was removed by two ether extractions. DNA was precipitated with 2 vols. of ethanol at 4°C, spooled and washed in 70% ethanol, resuspended in 1-2mL of TE and dialysed. Plasmid and cosmid DNA was prepared by a modification of the Birnboim and Doly method (Birnhoim, H. C. And Doly, J. (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA *Nucl. Acid Res.* 7:1513-1523. The volume of culture was 10nmL and the lysate was extracted with phenol/chloroform/iso-amyl alcohol before precipitation with isopropanol. Plasmid DNA to be used as vector was isolated on a continuous caesium chloride gradient following alkaline lysis of cells grown in 1L of culture. --.

Page 27, lines 22-32, delete in their entirety, and insert therefor

-- Colonies were screened for the presence of the O111 antigen by immunblotting. Colonies were grown overnight, up to 100 per

plate then transferred to nitrocellulose discs and lysed with 0.5N HCl. Polyoxyethylene(20)-sorbitan monolaurate (TWEEN 20) was added to TBS at 0.05% final concentration for blocking, incubating and washing steps. Primary antibody was *E. coli* O group 111 antiserum, diluted 1:800. The secondary antibody was goat anti-rabbit IgG labelled with horseradish peroxidase diluted 1:5000. The staining substrate was 4-chloro-1-naptol. Slide agglutination was performed according to the standard procedure. --.

Page 34, lines 6-25, delete in their entirety, and insert therefor

-- *E. coli* O157 O antigen gene cluster was amplified by using long PCR (Cheng et al. 1994, "Effective amplification of long targets from cloned inserts and human and genomic DNA" P.N.A.S. USA 91: 5695-569) with one primer (primer #412: att ggt agc tgt aag cca agg gcg gta gcg t (SEQ ID NO:5)) based on the JumpStart sequence usually found in the promoter region of O antigen gene clusters (Hobbs, *et al.* 1994 "The JumpStart sequence: a 39 bp element common to several polysaccharide gene clusted" Mol. Microbiol. 12: 855-856), and another primer #482 (cac tgc cat acc gac gac gcc gat ctg ttg ctt gg (SEQ ID NO:6)) based on the *gnd* gene usually found downstream of the O antigen gene cluster. Long PCR was carried out using the Expand Long Template PCR System from Boehringer Mannheim (Castle Hill NSW Australia), and products, 14 kb in length, from several reactions were combined and purified using the Promega Wizard PCR preps DNA purification System (Madison WI USA). The PCR product was then extracted with phenol

and twice with ether, precipitated with 70% ethanol, and resuspended in 40 µL of water. --.

Pages 94-103, renumber as pages 61-69, respectively.

IN THE CLAIMS:

Please amend the claims as follows:

Claim 85. (Amended) A method of testing a sample for the presence of *E. coli* expressing the bacterial polysaccharide O-antigen serotype 0111, the method comprising the steps of:

(a) providing genomic DNA of a sample to be tested;

(b) providing at least one oligonucleotide molecule, wherein said oligonucleotide molecule is at least about 10 nucleotides in length, and hybridizes using high stringent wash conditions to a nucleic acid sequence selected from the group consisting of:

wbdH (nucleotide positions 739 to 1932 of SEQ ID NO: 1);

wzx (nucleotide positions 8646 to 9911 of SEQ ID NO: 1);

wzy (nucleotide positions 9901 to 10953 of SEQ ID NO: 1); and

wbdM (nucleotide positions 11821 to 12945 of SEQ ID NO: 1),

wherein said high stringent wash conditions consists of 3 x 5 min washes in 2 x SSC and 0.1% SDS at room temperature, a 1 hr wash in 1 x SSC and

0.1% SDS at 58°C and 15 min wash in 0.1 x SSC and 0.1% SDS at 58°C;

(c) contacting said genomic DNA with said at least one oligonucleotide molecule to permit said oligonucleotide molecule to hybridize under said high strigent wash conditions to said nucleic acid sequence when present in said genomic DNA; and

(d) detecting any hybridized oligonucleotide molecules, wherein detection of said hybridized oligonucleotide molecules indicates the presence of said *E. coli* in said sample.

Claim 88. (Amended) The method as claimed in Claim 85, wherein said at least one oligonucleotide molecule is selected from the group consisting of positions 739-757 of SEQ ID NO:1, positions 925-942 of SEQ ID NO:1, positions 1165-1182 of SEQ ID NO:1, positions 8646-8663 of SEQ ID NO:1, positions 8906-8923 of SEQ ID NO:1, positions 9150-9167 of SEQ ID NO:1, positions 9976-9996 of SEQ ID NO:1, positions 10113-10130 of SEQ ID NO:1, positions 11821-11844 of SEQ ID NO:1, positions 12042-10259 of SEQ ID NO:1, positions 12258-12275 of SEQ ID NO:1, positions 1941-1924 of SEQ ID NO:1, positions 1731-1714 of SEQ ID NO:1, positions 1347-1330 of SEQ ID NO:1, positions 9908-9891 of SEQ ID NO:1, positions 9468-9451 of SEQ ID NO:1, positions 9754-9737 of SEQ ID NO:1, positions 10827-10807 of SEQ ID NO:1, positions 10484-10467 of SEQ ID NO:1, positions 12945-12924 of SEQ ID NO:1, positions 12447-12430 of SEQ ID NO:1 and positions 12698-12681 of SEQ ID NO:1.

Claim 89. (Amended) A method of testing a sample for the presence of *E. coli* expressing the bacterial polysaccharide O-antigen serotype 0157, the method comprising the steps of:

(a) providing genomic DNA of a sample to be tested;

(b) providing at least one oligonucleotide molecule, wherein said oligonucleotide molecule is at least about 10 nucleotides in length and hybridizes using high stringent wash conditions to a nucleic acid sequence selected from the group consisting of:

wbdN (nucleotide position 79 to 861 of SEQ ID NO: 2);

wbdO (nucleotide positions 2011 to 2757 of SEQ ID NO: 2);

wbdP (nucleotide positions 5365 to 6471 of SEQ ID NO: 2);

wbdR (nucleotide positions 13156 to 13821 of SEQ ID NO: 2);

wzx (nucleotide positions 2744 to 3109 of SEQ ID NO: 2); and

wzy (nucleotide positions 858 to 2042 of SEQ ID NO: 2),

wherein said high stringent wash conditions consists of 3 x 5 min washes in 2 x SSC and 0.1% SDS at room temperature, a 1 hr wash in 1 x SSC and 0.1% SDS at 58°C and 15 min wash in 0.1 x SSC and 0.1% SDS at 58°C;

(c) contacting said genomic DNA with said at least one oligonucleotide molecule to permit said

oligonucleotide molecule to hybridize under said high strigent wash conditions to said nucleic acid sequence when present in said genomic DNA; and

(d) detecting any specifically hybridized oligonucleotide molecules, wherein detection of said hybridized oligonucleotide molecules indicates the presence of said *E. coli* in said sample.

Claim 92. (Amended) The method as claimed in Claim 89, wherein said at least one oligonucleotide molecule is selected from the group consisting of positions 79-96 of SEQ ID NO:2, positions 184-201 of SEQ ID NO:2, positions 310-327 of SEQ ID NO:2, positions 858-875 of SEQ ID NO:2, positions 1053-1070 of SEQ ID NO:2, positions 1278-1295 of SEQ ID NO:2, positions 2011-2028 of SEQ ID NO:2, positions 2110-2127 of SEQ ID NO:2, positions 2305-2322 of SEQ ID NO:2, positions 2744-2761 of SEQ ID NO:2, positions 2942-2959 of SEQ ID NO:2, positions 5440-5457 of SEQ ID NO:2, positions 5707-5724 of SEQ ID NO:2, positions 13261-13278 of SEQ ID NO:2, positions 13384-13401 of SEQ ID NO:2, positions 861-844 of SEQ ID NO:2, positions 531-514 of SEQ ID NO:2, positions 768-751 of SEQ ID NO:2, positions 2042-2025 of SEQ ID NO:2, positions 1619-1602 of SEQ ID NO:2, positions 1913-1896 of SEQ ID NO:2, positions 2757-2740 of SEQ ID NO:2, positions 2493-2476 of SEQ ID NO:2, positions 2682-2665 of SEQ ID NO:2, positions 6471-6454 of SEQ ID NO:2, positions 5973-5956 of SEQ ID NO:2, positions 6231-6214 of SEQ ID NO:2, positions 13629-13612 of SEQ ID NO:2 and positions 13731-13714 of SEQ ID NO:2.

Claim 93. (Amended) A method of testing a sample for the presence of *S. enterica* expressing the bacterial polysaccharide O-antigen serotype C2, the method comprising the steps of:

(a) providing genomic DNA of a sample to be tested;

(b) providing at least one oligonucleotide molecule, wherein said oligonucleotide molecule is at least about 10 nucleotides in length and hybridizes using high stringent wash conditions to a nucleic acid sequence selected from the group consisting of:

wbaR (nucleotide positions at 2352 to 3314 of SEQ ID NO: 3);

wbaL (nucleotide positions 3361 to 3875 of SEQ ID NO: 3);

wbaQ (nucleotide positions 3977 to 5020 of SEQ ID NO: 3);

wbaW (nucleotide positions 6313 to 7323 of SEQ ID NO: 3);

wbaZ (nucleotide positions 7310 to 8467 of SEQ ID NO: 3);

wzx (nucleotide positions 1019 to 2359 of SEQ ID NO: 3); and

wzy (nucleotide positions 5114 to 6313 of SEQ ID NO: 3),

wherein said high stringent wash conditions consists of 3 x 5 min washes in 2 x SSC and 0.1% SDS at room temperature, a 1 hr wash in 1 x SSC and 0.1% SDS at 58°C and 15 min wash in 0.1 x SSC and 0.1% SDS at 58°C;

(c) contacting said genomic DNA with said at least one oligonucleotide molecule to permit said oligonucleotide molecule to hybridize under said high strigent wash conditions to said nucleic acid sequence when present in said genomic DNA; and

(d) detecting any specifically hybridized oligonucleotide molecules, wherein detection of said hybridized oligonucleotide molecules indicates the presence of said *S. enteria* in said sample.

Claim 96. (Amended) The method as claimed in Claim 93, wherein said at least one oligonucleotide molecule is selected from the group consisting of positions 1019-1036 of SEQ ID NO:3, positions 1708-1725 of SEQ ID NO:3, positions 1938-1955 of SEQ ID NO:3, positions 2352-2369 of SEQ ID NO:3, positions 2601-2618 of SEQ ID NO:3, positions 2910-2927 of SEQ ID NO:3, positions 3361-3378 of SEQ ID NO:3, positions 3578-3595 of SEQ ID NO:3, positions 3977-3994 of SEQ ID NO:3, positions 4167-4184 of SEQ ID NO:3, positions 4603-4620 of SEQ ID NO:3, positions 5114-5131 of SEQ ID NO:3, positions 5664-5681 of SEQ ID NO:3, positions 5907-5924 of SEQ ID NO:3, positions 6313-6330 of SEQ ID NO:3, positions 6697-6714 of SEQ ID NO:3, positions 6905-6922 of SEQ ID NO:3, positions 7310-7327 of SEQ ID NO:3, positions 7530-7547 of SEQ ID NO:3, positions 8007-8024 of SEQ ID NO:3, positions 1414-1397 of SEQ ID NO:3, positions 2170-2153 of SEQ ID NO:3, positions 2356-2339 of SEQ ID NO:3, positions 2759-2742 of SEQ ID NO:3, positions 3047-3030 of SEQ ID NO:3, positions 3311-3294 of SEQ ID NO:3, positions 3759-3742 of SEQ ID NO:3, positions 4378-4361 of SEQ ID

NO:3, positions 4774-4757 of SEQ ID NO:3, positions 5017-5000 of SEQ ID NO:3, positions 5515-5498 of SEQ ID NO:3, positions 6112-6095 of SEQ ID NO:3, positions 6310-6293 of SEQ ID NO:3, positions 6805-6788 of SEQ ID NO:3, positions 7068-7051 of SEQ ID NO:3, positions 7320-7303 of SEQ ID NO:3, positions 7775-7758 of SEQ ID NO:3, positions 7907-7890 of SEQ ID NO:3 and positions 8464-8447 of SEQ ID NO:3.

Claim 97. (Amended) A method of testing a sample for the presence of *S. enterica* expressing the bacterial polysaccharide O-antigen serotype B, the method comprising the steps:

(a) providing genomic DNA of a sample to be tested;

(b) providing at least one oligonucleotide molecule, wherein said oligonucleotide molecule is at least about 10 nucleotides in length and hybridizes using high stringent wash conditions to a nucleic acid sequence selected from the group consisting of:

wzx (nucleotide positions 12762 to 14054 of SEQ ID NO: 4); and

wbaV (nucleotide positions 14059 to 15060 of SEQ ID NO: 4),

wherein said high stringent wash conditions consists of 3 x 5 min washes in 2 x SSC and 0.1% SDS at room temperature, a 1 hr wash in 1 x SSC and 0.1% SDS at 58°C and 15 min wash in 0.1 x SSC and 0.1% SDS at 58°C;

(c) contacting said genomic DNA with said at least one oligonucleotide molecule to permit said oligonucleotide molecule to hybridize under said

high strigent wash conditions to said nucleic acid sequence when present in said genomic DNA; and

(d) detecting any specifically hybridized oligonucleotide molecules, wherein detection of said hybridized oligonucleotide molecules indicates the presence of said *S. enteria* in said samples.

Claim 100. (Amended) The method as claimed in Claim 97, wherein said at least one oligonucleotide molecule is selected from the group consisting of positions 12762-12779 of SEQ ID NO:4, positions 12993-13010 of SEQ ID NO:4, positions 13635-13652 of SEQ ID NO:4, positions 14059-14076 of SEQ ID NO:4, positions 14688-14705 of SEQ ID NO:4, positions 13150-13133 of SEQ ID NO:4, positions 13417-13400 of SEQ ID NO:4, positions 14051-14034 of SEQ ID NO:4, positions 14421-14404 of SEQ ID NO:4, and positions 15057-15040 of SEQ ID NO:4.

IN THE SEQUENCE LISTING:

Pages 61-93 (Sequence Listing), delete in their entirety.

REMARKS

In paragraph 3, on page 2 of the Office Action, the Examiner objects to the specification for reasons (a)-(b).

Specifically, with respect to reason (a), the Examiner states that a SEQ ID NO. needs to be included with respect to the sequences given at page 34.

Applicants hereby amend the specification as requested by the Examiner, and simultaneously file herewith a substitute Sequence Listing with respect to the same.

The Examiner is requested to note the substitute Sequence Listing (in PatentIn Version 3.1) is now considered to be a separate document by the U.S. Patent and Trademark Office. Hence, the present Sequence Listing in the above-identified application is not necessary and has been deleted from the specification.

Regarding reason (b), the Examiner states that at page 26, line 21 [*sic* line 18], "50mMTris-HCl" should read "50 mM Tris-HCl".

Applicants hereby amend the specification as requested by the Examiner.

In paragraph 4, on page 2 of the Office Action, the Examiner notes that the trademark TWEEN 20 is used in the specification, and states that such should be capitalized where ever it appears, and be accompanied by the generic terminology.

Applicants hereby amend the specification as requested by the Examiner, and also amend the first instance of the use of TWEEN 20 to recite its generic name "polyoxyetheylene(20)-sorbitan monolaurate" (see the relevant

page from the SciQuest Website which gives the generic name for TWEEN 20 (Appendix A)).

In paragraph 6, on page 3 of the Office Action, the Examiner rejects Claims 85-106 under 35 U.S.C. § 112, first paragraph.

Specifically, the Examiner states that the recitation "at least 10 nucleotides in length" is not supported in the specification, i.e., the Examiner contends that the specification (see page 10, and original Claim 5) only supports "about 10 to about 20 nucleotides".

In view of the amendments to the claims to recite "at least about 10 nucleotides in length", Applicants respectfully submit that the Examiner's rejection has been rendered moot".

In paragraph 7, on page 3 of the Office Action, the Examiner states that there is no support in the specification for "*weaR*" as recited in Claim 93, although the Examiner states that there is support at page 11 for "*wbaR*".

Applicants hereby amend in Claim 93, to recite "*wbaR*", as requested by the Examiner.

In paragraph 9, on page 4 of the Office Action, the Examiner states that the method of Claim 101 requires one to perform hybridization reactions between a probe of undefined sequence to a target sequence found in any of a variety of "sugar pathway genes".

The Examiner contends that the specification fails to provide an adequate written description of the coding sequence for these genes as found in any of the encompassed microorganisms, much less an adequate written description of the

probes and primers that would be used in the hybridization and detection aspects of the method.

For the following reasons, Applicants respectfully traverse the Examiner's rejection.

Applicants respectfully submit that the sequences of oligonucleotide molecules which hybridize to the "sugar-pathway genes specific to the bacterial strains to be detected" were well-known to one skilled in the art, and/or ones skilled in the art would have been able to identify oligonucleotide molecules capable of hybridizing thereto without undue experimentation (see the last paragraph at page 6 of the present specification).

Moreover, Applicants submit herewith a list of citations dated prior to 1997 (Appendix B) and copies of related references (Appendix C) which disclose a range of genes associated with sugar biosynthetic pathways. These citations show that sugar pathway genes were well-known in the art, and formed part of the common general knowledge. As a result, one skilled in the art would have been able to use her common general knowledge and routine techniques available in the art to produce oligonucleotide molecules capable of hybridizing to such genes.

Accordingly Applicants respectfully submit that the claims clearly and definitely recite the invention of interest, and are enabled by the present specification. Thus, the Examiner is requested to withdraw the rejection.

In paragraph 11, on page 4 of the Office Action, the Examiner rejects Claims 85-106 under 35 U.S.C. § 112, second paragraph.

Specifically, the Examiner states that the claims are indefinite in what constitutes "specifically hybridize".

The Examiner is requested to note that that "specific hybridization" occurs under "high stringent" conditions. Thus, Applicants hereby amend the claims to recite to recite the hybridization conditions as being high-stringency washing as described at page 28, lines 19-22, and to amend "specifically hybridizing" to "hybridizes under high stringent wash conditions", thereby rendering moot the Examiner's rejection.

In paragraph 13, on page 4 of the Office Action, the Examiner contends that Claim 101 is indefinite with respect to which genes are considered to constitute "sugar-pathway genes".

As discussed above, "sugar-pathway genes" were well-known to one skilled in the art, and thus this expression is not indefinite.

In paragraph 14, on page 5 of the Office Action, the Examiner states that Claims 88, 92, 96 and 100 are confusing with respect to which "oligonucleotide molecule" Applicants are referring to as found in any of the recited tables.

Applicants hereby amend Claims 88, 92, 96 and 100 to specifically recite the oligonucleotides from the tables that are of interest, and not refer to the tables in the claims, thereby rendering moot the Examiner's rejection.

In paragraph 15, on page 5 of the Office Action, the Examiner rejects Claims 85-106 under 35 U.S.C. § 112, second paragraph.

Specifically, the Examiner states that these claims omit the essential step of (a) use of a detectable moiety in

conjunction with the hybridization reaction; and (b) assuming that the claims are amended to require a detectable moiety, the claims do not recite any step whereby unbound/non-hybridized oligonucleotides would be removed from the assay solution so that only the hybridized sequences would be detected.

For the following reasons, Applicants respectfully traverse the Examiners rejection.

While the PCR products would be detected, the detection means is not critical to the present invention. Thus, it is improper for the Examiner to require Applicants to limit the claims to a specific detection means.

Accordingly, Applicants respectfully submit that the claims clearly and definitely recite the invention of interest, and thus request withdrawal of the Examiner's rejection.

In view of the amendments to the specification and claims and the arguments set forth above, reexamination, reconsideration and allowance are respectfully requested.

The Examiner is invited to contact the undersigned at his Washington telephone number on any questions which might arise.

Respectfully submitted,

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